Regional Activity to Promote Integration Through Dialogue and Policy Implementation (RAPID)

Heartwater Disease Research Project Review

Task Order No. 4.2

Task Order Technical Report No. 1

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LIST OF ACRONYMS

BCR	Benefit Cost Ratio
GMP	Good Manufacturing Practices
HW	Heartwater Disease (*Cowdria ruminantium*)
ILRI	International Livestock Research Institute, Nairobi, Kenya
PCR	Polymerase Chain Reaction
RAPID	Regional Activity to Promote Integration Through Dialogue and Policy Implementation
RCSA	Regional Center for Southern Africa
SADC	Southern Africa Development Community
TO	Task Order
UF	University of Florida, Gainsville, Florida
USAID	United States Agency for International Development

EXECUTIVE SUMMARY

Livestock production in sub-saharan Africa is burdened by various diseases and their insect vectors. Heartwater disease, (*C. ruminantium*) carried by amblyomma ticks is a significant problem in the southern African region where 65% of livestock are raised in amblyomma tick-infested areas. Commercial and small holder livestock owners have to constantly deal with HW production losses and pay for preventative techniques including frequent acaricide application. It is estimated that vaccination against HW would avoid US $97 million in losses and acaricide costs annually in the SADC region. After starting in 1985 under an USAID funded project, scientists at the University of Florida in Gainsville, Florida and in Harare, Zimbabwe have done research resulting in an inactivated vaccine against HW disease. This research has yielded other promising results such as recombinant DNA vaccine, HW diagnostic technologies and a tick-decoy innovation which attracts and kills amblyomma ticks on animals. Some of the research results are nearing readiness for commercialization, but additional research is needed to make them more attractive to the private sector.

The purpose of this review under RAPID Task Order 4.2 was to provide a basis for USAID decision-making on the future of this long-standing HW research project. The consultants assessed the status of the research as it related to the results being of potential interest to the private sector. We were requested to provide guidance and options for future USAID support and to present strategies to move project results toward commercial takeover and marketing. If this were achieved, USAID's investments would yield long-term, beneficial contributions to livestock production in the southern African region.

The review concluded that the project had made impressive scientific accomplishments, including the establishment of an outstanding laboratory capability in Harare, but was insufficiently focused on the results being made attractive for commercialization. To realize this goal, several additional studies are needed on specific attributes of the inactivated and the DNA HW vaccines. The option for USAID support to enable this research to be conducted by September 2001 has recently been granted. Further trials on the tick decoy to demonstrate effectiveness against other tick species would increase private sector interest in this innovation. To accomplish this tick-decoy research, USAID has the option to grant funding for September 2001-September 2002. Exercising this option could also include funds to complete duration-of-immunity trials for the inactivated vaccine. This vaccine attribute appears to be a significant requirement for private sector interest in licensing agreements with UF.

Because of the significant benefits the tick-decoy technology holds for all of Africa, we recommend that the planned trials be given as much support as possible, unless negotiations with the private sector result in their taking over this research. In order to assess and explore the commercial attractiveness of the inactivated vaccine, we recommend that Task Order 4.2 initiate and fund meetings in the near future between UF staff and officers of veterinary biologic companies. The aim of these meetings would be to establish arrangements to effect the transition of the scientific research to the private sector.

SECTION I – INTRODUCTION

1. Purpose of RAPID Task Order 4.2

The overall purpose of this task order is to provide a basis for decision making to determine the future of the long-standing USAID initiative to fund research on heartwater (HW) disease prevention in southern Africa. This project is administered by the University of Florida in Gainesville, FL, where scientists did the preliminary work and continued laboratory activity on the inactivated and recombinant DNA vaccines against HW. Field testing and applied research on the vaccines was done at the Veterinary Research Laboratory in Harare, Zimbabwe. The vaccine research on the potential products are now at a point where they are nearing readiness for private sector commercialization. Another important research result is the specification of diagnostic methods to detect the HW organism in animals and ticks. Post graduate training for African students was accomplished at UF and in Zimbabwe. The project funded several valuable epidemiologic and economic studies through subcontracts with staff at the International Livestock Research Institute (ILRI) in Nairobi in conjunction with the University of Warwick, UK.

Another problem in preventing HW is the control of the tick vector. Workers at UF, in Zimbabwe, and in the Caribbean island of Guadaloupe, have designed and tested a tick-decoy technology (a plastic tag or collar which attracts and kills ticks on the host animal). Implementation of this tick decoy would enable farmers to avoid the costly, time-consuming practice of applying acaricides to their cattle, sheep and goats on a frequent basis. After some additional field testing, this innovation will hold potential for private sector development under license agreements with UF.

This task order was designed to yield results that directly contribute to RCSA's Strategic Objective 4 – *The commercialization of agricultural technologies to private markets within the SADC region,* and to Intermediate Result 4.3 – *private sector participation to increase delivery systems of improved agricultural technologies.* This study will assist in defining near-term research needs and in identifying prospects for subsequent commercialization.

2. Objective of this Review

The basic objective of this review was to assess the current status of project research on the inactivated HW vaccine, the recombinant DNA HW vaccine and the tick decoy as this status relates to the research results being potentially attractive for commercialization by private sector enterprises. We were requested to provide guidance and options for future USAID support of this research and to present strategies to move the project research results toward takeover by private sector so that USAID's investments would yield long-term, beneficial contributions to livestock production in southern Africa.

3. Organization of the Report

The four parts of the main body (Section II) of this report address the topics below. The observations described for these topics formed the basis of our conclusions and recommendations on options for future USAID decisions and strategies.

A. The HW infection - tick vector problem and its economic consequences.
B. Research results and potential products.
C. Overall view of research accomplishments.
D. Realizing the commercial potential of research discoveries.

4. Consultants' Work Activities

Following initial preparation and research document review, we first visited scientists at UF. Then we carried out field visits and interviews in southern Africa during March 2001. There we visited and interviewed livestock farmers and veterinarians having first-hand experience with HW problems in Zimbabwe, Eastern Cape and Northern provinces of South Africa and in Botswana. We investigated several project research issues in detail with staff at the Veterinary Research Laboratory facilities in Harare. Also we talked with officials of animal health product companies in South Africa, Botswana, Zimbabwe, France, the Netherlands and the U.S.

The consultants for this project review were E. Hunt McCauley, DVM/Agricultural Economist, Al Strating, DVM/former director of APHIS Centers for Epidemiology and Animal Health;, and Tim Skinner, livestock pharmaceutical marketing specialist. The team worked under the supervision of the RAPID Agricultural Advisor, Susan Corning.

SECTION II – FINDINGS AND ANALYSIS

A. THE HEARTWATER INFECTION-TICK VECTOR PROBLEM AND ITS ECONOMIC CONSEQUENCES

A1. The Heartwater Disease Burden For Livestock Production

In areas at risk for HW, livestock owners deal with a complex interplay between the infection itself and the tick vector. Beyond having to treat clinical cases, these owners are in a constant battle to prevent the occurrence of HW infection and/or attempt to provide a degree of immunity so the infection results in fewer adverse clinical outcomes in their animals. This battle requires burdensome management practices and costly tick control procedures. (See Annex A for a detailed description of HW disease and its vectors.)

The burden for commercial cattle and sheep farmers producing improved breeds appears to be greater or more complex than for small holders/traditional owners whose livestock graze on communal land and are generally indigenous types which have a certain degree of resistance to illness from HW infection. Endemic (enzootic) stability is the situation where, due to constant challenge by HW infected ticks, the animals develop an acquired immunity for HW disease. The ticks are simultaneously the "infectors" and "vaccinators". So, due to lack of any other immunization technology, farmers will use acaricides strategically depending on the tick loads they observe on their animals. For example, it is not desirable to have animals completely free of ticks all of the time because this decreases the opportunity for natural HW challenge. The maintenance of the endemic stability status is complicated by several unknowns including, "Are the tick vectors actually carrying the HW organism?" and "What is the optimal acaricide application frequency to achieve immunity?" This immunity is not perfect and some tick-challenged animals show clinical HW signs and have to be treated.

Another burdensome control practice is the prophylactic antibiotic treatment during the tick season of young, unexposed animals and "naive" animals moved from tick-free zones to at-risk zones. Farmers find they have to give this treatment frequently, every 10-18 days, during the tick season, otherwise animals die from the untreated infection. Generally, in tick-infested areas such treatment is required up to 6 months of age at which time they may have sufficient immunity to protect from the HW challenge in their environment, but this depends on the severity of the tick season. Angora goats and merino sheep are particularly susceptible to HW, so that owners have to “block” with antibiotics every two weeks. Even under this demanding regime, owners still lose a few, like one percent, but without this treatment they experience 10 to 20 percent mortality losses.

Another, but more complex approach to establishing immunity, is the injection of blood from HW infected sheep produced by the Veterinary Institute in Onderstepoort, South Africa. This practice is cumbersome because the blood product has to be kept in liquid nitrogen until it is injected intravenously. After the injection, animals must be monitored until they show a temperature rise at which time they are given antibiotics to "block" the illness, but allow immunity to be established. This so-called "blood and block" procedure is risky and indeed, some animals die following injection even with the treatment.

Onderstepoort produces about 120,000 doses per year of this virulent blood, most of which is sold in South Africa to commercial producers. Some of it is used in Botswana for a government veterinary service program, under which farmers who bring cattle from the tick-free, western regions to the tick-infested, eastern region, pay a subsidized fee for the "blood and block" procedure. In our many interviews with farmers and veterinarians, they described the need for a vaccine to be almost "desperate". Aside from the HW related production losses, the problems farmers deal with on an almost weekly basis of treating or preventing HW infection and exercising optimal tick control cause significantly increased operating costs.

A2. Economic Consequences

The scope of the potential economic impact is extensive. Some 65% of the livestock in the SADC region are raised in amblyomma-infested areas, and therefore, at risk for HW infection. Economic evaluations of this impact and its reduction through the use of vaccines were estimated for nine countries by Minjauw and Mukhebi in collaboration with veterinary officers in each country and with colleagues at ILRI in 1998. Selected results of these estimates are summarized in Table A-1. Estimates of the impact of animal disease are difficult under the best of conditions but particularly for this disease in the setting of southern Africa. Dr. Minjauw describes the nature of this difficulty:

> "A study of the economic impact of a disease such as heartwater and of its potential control through the use of new vaccines in the future, is a complicated task. There are many reasons for this including the varying data availability and quality issues, quite apart from the logistical issues of identifying, collating and evaluating data from the different production systems in the country. In addition, it is important to not only estimate the total production losses and control costs, but also to evaluate and quantify the avoidable losses that result from a planned intervention programme, in this case the use of new inactivated heartwater vaccines. These difficulties are further compounded by the complexities of *ex ante* impact assessments of a new technology not yet available for farmer evaluation, as well as possible regional issues associated with vaccine production and distribution that could affect the availability and impact of new vaccines."

The authors of these studies had to make a variety of assumptions on issues such as: accuracy of diagnosis, types of production losses due to HW and the use of acaricides against amblyomma ticks as compared to use to control ticks which transmit other diseases and to prevent the direct physical effects of tick attachment ("tick damage"). In some countries with high incidence of East Coast Fever, anaplasmosis and babesiosis, the use of acaricides would remain high regardless of a possible reduction of their use due to adoption of an effective HW vaccine. As shown by the low "acaricide costs-avoided" in Table A-1, Tanzania is such a country according to these estimates. Also, they had to estimate the adoption rates of a vaccine by different production systems – ie. commercial and smallholder or communal farmers in the different countries. Another issue was the price of the vaccine and its duration of immunity, both of which are unknown at this point.

The authors calculated discounted, benefit-cost ratios (BCR's) over 10 years, for the different types of livestock systems: commercial operations and small holder/traditional/communal production. The benefit stream was the morbidity and mortality production losses and treatment costs avoided, plus savings from reduced acaricide use attributable to HW vaccination. The costs to achieve these benefits were for the vaccine and service. The estimates included assumptions of adoption rates, and duration of

immunity. The baseline cost of the vaccination was estimated from the East Coast Fever vaccine cost in Zimbabwe of $3.30 US per vaccination. These BCR's varied from country to country but most greatly between commercial and small holder systems. The highest rates of return were estimated for commercial dairy cattle and angora goat production.

The small holder/traditional sector would seem to benefit least under the assumptions used for losses avoided by vaccine use. This is not because the small holder livestock farmers do not suffer production losses, but most probably because they treat clinical cases infrequently due to the antibiotic cost and operate in an environment of endemic stability. Also, they use acaricides less often or not at all in some settings, so that "costs-avoided" benefits are estimated to be very low. Many do not sell livestock but deal in barter trade and bride-price arrangements; so their livestock production/loss values difficult to verify.

The authors further estimated "breakeven" prices for the vaccine and its administration under the various scenarios (e.g., small holder and commercial). The highest breakeven prices were estimated for commercial dairy cattle and angora goat production in South Africa ($11.90 and $4.30 respectively), while this estimate for small holder/traditional production was generally less than $1 in all the countries. Bear in mind that data on smallholder production output and costs is less reliable than data for commercial farmers who produce livestock under relatively more intensified practices.

A3. Potential for Vaccine Use in the SADC

Certainly for the commercial livestock production systems in at-risk areas, the potential use of inactivated HW vaccine is high. Vaccination would quickly find utility in several situations as indicated in the previous discussion and in Annex A.

1. For young livestock that are presently being immunized by the "blood and block" technique or by allowing natural challenge coupled with antibiotic treatment.
2. For naïve (non-immune) animals of all ages moving to an enzootic area such as happens frequently in Botswana.
3. For animals in amblyomma-free status areas if this status changes when the ticks start to appear in these areas. This can occur after introduction of wild ruminants, as has been noted in Zimbabwe; or a climactic change to more wet weather conditions favorable to tick activity.

The adoption of a vaccine by small holder/traditional farmers may be predictably less, but they do suffer HW production losses, particularly in young animals. As derived from recent anecdotal evidence, small holder demand likely exists for the vaccine. In fact, because the traditional system holds some five times the livestock held by commercial farmers, even a low percentage of adoption would likely lead to an equivalent demand quantity.

Furthermore, the additional benefit of improved environmental conditions due to reduced use of toxic insecticides and acaracides could be expected with prevention of these diseases by immunization rather than continuous tick control. This benefit would, of course, be further enhanced by application of the tick-decoy technology.

Table A-1: Estimated Heartwater Impact Avoided By Vaccine Use [1]

COUNTRY	COMMERCIAL CATTLE AT RISK ['000's]	COMMERCIAL SHEEP & GOATS AT RISK [' 000's]	TRADITIONAL CATTLE AT RISK ['000's]	TRADITIONAL SHEEP & GOATS AT RISK [' 000's]	TOTAL ANNUAL PRODUCTION AND CONTROL COSTS AVOIDED [US$ million]	ANNUAL ACARICIDE COSTS AVOIDED [US$ million]
SOUTH AFRICA	3,190	4,010 [2]	1,718	2,717	31.6	10.7 [34%]
TANZANIA	271	20	15,374	14,167	2.9	0.2 [7%]
ANGOLA	175	34	3,325	1,685	0.8	0.1 [17%]
ZAMBIA	1,040	121	1,700	673	3.7	1.4 [38%]
ZIMBABWE	1,065	485	2,578	689	5.6	4.3 [76%] [3]
BOTSWANA	119	30	718	1,368	0.4	0.1 [25%]
SWAZILAND	146	31	495	428	1.9	1.6 [86%]
MALAWI	28	18	592	1,681	0.3	0.1 [33%]
MOZAMBIQUE	61	20	291	519	0.3	0.1 [33%]
TOTAL	**6,095**	**4,769**	**26,791**	**23,927**	**97**	**18.6 [39%]**

1. Summarized from draft country reports done by B. Minjauw and colleagues at ILRI and University of Warwick, 1998, and from the A.W. Mukhebi, etal. assessment of impact of heartwater in Zimbabwe. Prev. Vet. Med. 1999.
2. Mainly angora goats in East Cape Province.
3. In Zimbabwe all acaricide costs were assumed to be related to HW prevention through amblyomma tick control. In other countries, a portion of acaricide usage was assumed to be related to prevention of other disease and tick damage and therefore, would be continued in varying degrees.

B. RESEARCH RESULTS AND POTENTIAL PRODUCTS

At an international conference in Orlando, Florida in 1983, two accomplished experts on tick borne diseases in Africa, Drs. Mike Burridge and Andy Norval, engaged in a spirited discussion on the subject of heartwater disease in sub-Saharan Africa and the damage that it caused animal agriculture in the region. The need for new methods to control the disease was very apparent to both scientists, and there was no shortage of ideas about potential ways of addressing the problem. The direct result of these discussions was a request for funding through the University of Florida for research directed at developing control methods. USAID funding was first authorized in 1985.

Over the succeeding years, the project has made significant progress in several areas. An inactivated vaccine has been discovered which provides a significant degree of protection against heartwater losses. Also a DNA vaccine, while not as far along in research, has shown early promise. A product called a "tick decoy", containing an acaricide and a pheromone attractant has been successfully tested in Africa and the Caribbean. Finally, a polymerase chain reaction (PCR) assay developed by the project is in use and is a major advance in providing needed definitive diagnostic capability.

B1. Inactivated Heartwater Vaccine

Immunity to HW is complex. The exact mechanisms by which resistance to disease is effected after vaccination or after natural exposure to the organism are not completely understood. It is clear that serum antibody levels play a relatively minor role in immunity. Passive transfer of immune serum to an exposed animal has no beneficial effect on the course of the disease. Since *Cowdria ruminantium* is an obligate, intracellular organism, cell mediated immunity would be expected to play a major role in protection from disease. While T- cells have been clearly shown to be involved in protection, much remains to be learned about the role of specific T-cell subpopulations, interferons, and other immunochemicals.

Following either vaccination or naturally occurring infection, long lasting immunity to re-infection does not appear to consistently develop. Indeed, in the field the whole concept of endemic stability is based on the need for repeated re-exposure to the organism in order to maintain continuing high levels of resistance to severe disease. Vaccine trials using a wide variety of approaches and antigens also have provided significant protection from death losses but never complete resistance to infection. It seems clear that development of effective vaccine against heartwater will never be as uncomplicated as those against which, for example, resistance is mediated through development of protective serum globulins.

A crude form of vaccination against HW has been carried out for decades in southern Africa. It is referred to as the "blood and block" method as described in part A of this section and Annex A. In 1991 a live attenuated heartwater vaccine was described, using the Senegal strain which had been modified after only a few serial passages in cell cultures. While the vaccine was safe and provided protection against homologous challenge, it did not protect well against challenge with isolates of geographically diverse backgrounds.

The USAID funded HW project scientists at the University of Florida have been leaders in the research of inactivated vaccine. Improvements in the methods of growing the organism in cultured endothelial cells has greatly facilitated the production of large quantities of antigen. The establishment of the HW research facilities in Harare,

Zimbabwe has been pivotal, since no work with the live organism can be conducted in the United States, where the disease is considered exotic.

The production of the inactivated vaccine involves conventional, uncomplicated procedures. Methods have changed little since the first vaccines were produced and tested almost a decade ago. The vaccine is grown on monolayers of "primary" endothelial cells, harvested after infection is complete, and after certain interim procedures, is inactivated with beta propriolactone, a widely used agent.

Throughout the past 10 years, efforts to refine, improve and test the vaccine have continued. In various trials in cattle, sheep, and goats, for efficacy against lethal challenge, protection ranging from 60-70% was usually realized. Early trials used a vaccine made from a strain of the agent called Crystal Springs, together with Freund's adjuvant. Subsequently, it was shown that the Mbizi strain of the agent provided a much superior range of cross protection against other geographically diverse isolates. Also, since complete Freunds adjuvant is not acceptable for commercial use, the search for a new adjuvant resulted in the use of Montanide ISA 50, an acceptable commercially available adjuvant. A number of studies have confirmed the efficacy and safety of vaccine produced using the Mbizi strain and the Montanide ISA50 adjuvant.

A large field trial was recently completed in four southern Africa countries. (Botswana, Republic of South Africa, Zambia, and Zimbabwe). The Mbizi/Montanide ISA50 vaccine was used in each country, plus vaccine prepared from local isolates of the organism. In Botswana, Zambia, and Zimbabwe the Mbizi strain provided better protection than the vaccine prepared from local strains. In the Republic of South Africa, however, angora goats were more effectively protected using the local Bathurst strain than by the Mbizi vaccine. It should be noted that angora goats are extremely susceptible to HW and represent a unique situation.

B1a. Further Research – Inactivated Vaccine

The field trials and other recent work suggest the need for a number of additional studies of the vaccine. While it is known that the Mbizi strain of the organism produces highly cross reactive protection, the results in angora goats indicate the need for a bivalent vaccine or the production of a separate vaccine for angora goats or in other unique situations. Secondly, while Montanide ISA50 seems to be an excellent adjuvant, five additional adjuvants have recently been made available for test and might provide better vaccine efficacy. Tests to determine the optimal age of vaccination have not been conducted and the duration of immunity after vaccination has not been determined. In addition, a number of less expensive studies must be conducted for the vaccine to be considered fully characterized. Some of this research is now in progress and will be completed by May-June 2001 which would enhance the attractiveness of the vaccine for commercialization.

B2. DNA Vaccines

In addition to the work on conventional inactivated vaccines, the project has expended considerable effort toward the development of gene-based vaccines. Molecular biologists at the University of Florida have worked collaboratively with scientists from a number of other locations including Washington State University, the ILRI, and the project's Heartwater Research Laboratory in Harare. The work has produced results that are beyond the proof-of-principle stage and moving toward testing vaccines in target animals.

Early indications of promise were realized with DNA vaccine containing the gene for the major antigenic protein 1 (Map 1) of *C. ruminantium*. The vaccine was constructed by cloning the Map1 gene into a commercially available expression vector. Following intramuscular injection into small laboratory animals (mice), immune responses were demonstrated by several measures. Further, vaccinated mice were significantly protected against lethal challenge.

Subsequent experiments have shown that responses in mice could be enhanced by also giving a Map 1 protein boost following the initial DNA vaccine injections. This approach also provided some protection against challenge with geographically diverse strains of the organism. Project researchers have identified a number of additional genes that code for proteins that are recognized by antibody and peripheral blood mononuclear cells from known immune cattle. Currently, both sheep and cattle have been vaccinated with DNA vaccine followed by protein boosts. In addition, newly published reports of intra-lymphatic inoculations of DNA vaccines are being evaluated. Immune responses and resistance to lethal challenge with *C. ruminantium* will be determined. Research on MAP-1 DNA vaccines in target animals is presently ongoing in Zimbabwe and will be completed in July 2001. A "cocktail" DNA vaccine trial of six genes of HW in sheep will start in May and be completed by September 2001.

The project's work on DNA vaccines has contributed much to understanding the molecular biology of *C. ruminantium*. Results in laboratory animals have been encouraging. It should be pointed out, however, that despite accelerated efforts in recent years, there have been no naked-DNA vaccines taken from the laboratory to commercial success. The reasons for this remain unclear, but some have suggested that it involves the size of the target host and the volume of DNA required to effect immunity. A number of vaccines, primarily intended for humans, have shown good protection of small laboratory animals but failed to show adequate efficacy when tested in primates or other large target species.

DNA vaccines are generally considered to be effective producers of T cell responses, at least as compared to their ability to elicit serum antibody responses. This fact would favor prospects for them being effective against heartwater. In addition, DNA vaccines would appear, at least conceptually, to be amenable to providing a broad range of protection against the many heartwater strains that exist, as well as against other tick-borne diseases that exist in southern Africa. Such a broadly protective product would naturally provide huge benefits as compared to individual vaccines against diseases, or compared to the availability of vaccines against only one or two of the tick-borne diseases.

B3. Tick Decoy

Tick control has traditionally been the major means by which heartwater, as well as a number of other tick- borne diseases, has been controlled. Throughout much of the 20th century this has been done through the use of acaricides, typically applied by dipping, spraying, or pouring the products onto the animal. As described in the previous section A, these procedures are costly, cumbersome, environmentally unfriendly, and require constant attention. Because large quantities of acaricide are required, particularly in the immersion tanks, residues and disposal problems are challenging for both large and small livestock producers. The cost of purchasing these quantities of acaricide from foreign suppliers became prohibitively expensive, and as a result, less and less tick control was available, particularly for the small holder sector. Moreover, even when widely used,

these procedures have not proven to be very effective in controlling the problem. The need for tick control methods that are effective, inexpensive and environmentally friendly has long been recognized.

The early versions of the tick decoy consisted of a plastic tail tag that had been impregnated with an acaridide, plus a pheromone attractant. This ingenious device would attract ticks to the animal where the acaricide would kill them. A 1996 publication describes a large field trial conducted in Zimbabwe in which impressive efficacy was demonstrated. While some variability in results was noted, parts of the trial demonstrated bont tick control at levels greater than 95%.

Recognizing that a number of other species of ticks cause severe problems for livestock, and that some of these have an affinity for the head and ears, a neck collar application has also been developed. A large trial in the Caribbean was subsequently conducted using both the tail and collar applications. Again, significant levels of efficacy were demonstrated. While retention of both the collar and tail tags has been good, they are brightly colored so that losses can be easily noted. Duration of efficacy of up to three months has been demonstrated, although some variability in the stability of the products has been seen and associated with variations in ambient weather conditions. The tick decoy is expected to allow a certain small number of ticks to survive on livestock, so endemic stability will probably be maintained. It could be especially attractive for use by small holders, since it would be relatively inexpensive and easy to apply, particularly as compared to traditional methods of acaricide use

Before the tick decoy can be considered ready for commercial acceptance, field trials must be conducted in Africa using currently available acaricides in the decoy and both the tail and neck collar applications. An important objective is to evaluate the effectiveness against ticks other than amblyomma species. Approval to conduct field trials has been obtained in the Republic of South Africa (RSA) and Zimbabwe, but these must await the beginning of the next tick season in October 2001. Presently, in Zimbabwe a preliminary, small trial using tick-decoy collars is underway and will be completed by the end of May 2001.

B4. Diagnostic Test Methods

At the onset of the heartwater disease research project, there were no simple and reliable methods for diagnosing the disease. As a result, little was known of the incidence, prevalence and epidemiology of the disease. In the context of the relatively broad mandate for developing strategies for controlling HW, great effort was directed, particularly in the early years, toward finding reliable diagnostic methods. Much progress has been made, some in collaboration with other institutions, but largely through the work of project scientists. This is true despite the fact that, the organism itself, and the animal's immune response to it are both very complex.

In developing better methods for assay of immune responses to either naturally occurring infections or vaccination, incremental improvements have been made. The work was complicated by the presence in the field of a number of closely related disease organisms that are very closely related to *C. ruminantium*. Antibody to these organisms tends to cross-react with the HW antigens. The latest serological test developed by project scientists, with the collaboration of the University of Utrecht, is called the MAP-1B ELISA. While it is not yet a perfect serologic test, it is far superior to previous methods.

In detection of *C. ruminantium*, the organism itself, in either ticks or in host animals, the traditional methods were limited to small animal inoculation for detection in ticks, or in the case of ruminants, to microscopic examination of brain smears obtained either by biopsy from a living animal or at necropsy from one that had died. Again, project scientists have been central to development of vastly improved procedures. Development of first a DNA probe to detect *C. ruminantium* antigens, followed by the development of a clearly superior PCR assay has provided an invaluable tool to diagnosticians, epidemiologists and animal health officials. Rapid, accurate detection of the agent itself is particularly important in following the movement of the disease into previously uninfected areas. For example, in the United States, which remains free of HW but is at increasing risk due to its presence in the Caribbean, the availability of an accurate, fast and sensitive assay is vitally important. Recent trials by project scientists have shown the PCR assay to be superior to other available methods in both sensitivity and specificity and diagnosticians from the U.S. Department of Agriculture were trained at the HW project laboratories in Harare in the use of the PCR assay.

C. OVERALL ASSESSMENT OF RESEARCH ACCOMPLISHMENTS

In assessing the quantity and quality the HW disease research conducted through the University of Florida over the course of their association with USAID, one must surely be impressed. A total of 169 full-length, scientific publications have documented the results of work that covered a wide range of disciplines, including microbiology, immunology, molecular biology, parasitology, epidemiology, and a number of others.

Major advances in understanding the molecular biology of this complex organism have been largely attributable to the work of project scientists. The immune responses to the organism are very complex, but are now understood to a much greater degree than they were when the project began. The availability of much improved diagnostic methods represents a major set of accomplishments that have great value. Further, even if the vaccines and the tick decoy were never to be licensed for commercial production, these accomplishments provide a body of scientific data on which further efforts can be based.

The formation of the HW research project laboratories in Harare is a remarkable accomplishment in itself. Heartwater work could not, of course, be conducted using the live organism in the U.S., so the establishment of a facility in a location where the disease is endemic was essential. This facility has capabilities that range from molecular biology to the efficient raising of laboratory animal colonies. The staff is competent and dedicated, though many have learned only through experience or been tutored by exceptional laboratory leadership. It is a remarkable resource, not only for HW research, but also to Zimbabwe and the entire southern Africa region for investigation of other animal diseases.

Some criticism has been directed at the project relating to the lack of commercialization of products that have resulted from the research. In our opinion, this would appear to be at least partly justified. As early as 1991, the Technical Advisory Group urged USAID and the project to focus on getting the results of the research into commercial channels as soon as possible. Specifically, they recommended the addition of staff expertise in product registration. Now, ten years later, it still has not happened. Historic files indicate that USAID did not aggressively push the project toward the registration of commercial products until recent years. At the same time, it is the natural inclination of researchers to direct their efforts toward pure research and not toward commercial success. In summary, the research was excellent but at times appeared to lack commercial focus, at least as defined in the later opinion of the funding agency.

D. REALIZING THE COMMERCIAL POTENTIAL OF RESEARCH RESULTS

This part of the report sets out to outline the model used in moving a product from its initial investigation/experimental phase through to a marketable solution, within the framework of a commercial company. Using this model as a benchmark, the current status of each of the three research results is then assessed, and positioned, based on their readiness for commercialization. This state of readiness has a direct impact on a potential product's attractiveness and relative value to any commercial company. Consequently it determines the approach required in achieving the transition from an USAID funded research program to commercially funded product development initiative.

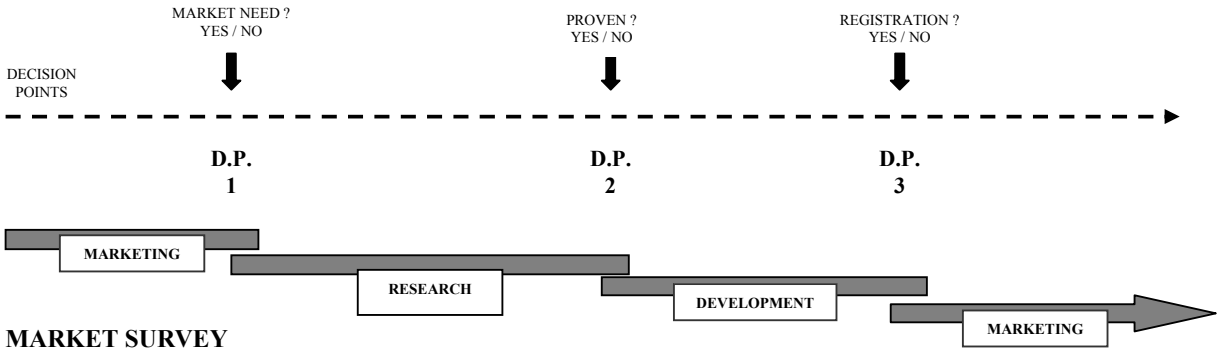
D1. The Commercialization Pathway

Typically within a commercial company, the progression of a product from a scientific research result to marketing, is the responsibility of three distinctly separate, but interactive teams:

- Primary researchers
- Product developers
- Marketing team

Each of these groups is responsible for key "Go / No Go" decisions along the continuum of a products evolution. This is shown in Figure D1 below.

Figure D1. Typical Pathway to the Commercialization of a Potential Product



MARKET SURVEY

- Identify problem
- Determine size of market
- Formulate concept

PROOF OF THEORY/CONCEPT

- Production methodology
- Efficacy in target animals
- Cross protection
- Duration of immunity

PREPARATION FOR REGISTRATION

- Production procedures [GMP]
- Patent registrations
- Stability data
- Pharmacology
- Toxicology data
- Residue data
- Establish claims and label

PREPARATION FOR LAUNCH

- Commercial literature
- Packaging
- Interest groups meetings
- Set up of distribution pathway
- Launch

D1a. Assessment of the Market Requirement

The assessment of the need for a solution to a problem in the market and therefore the potential for developing a marketable solution has traditionally been the responsibility of the marketing team. However, as the rate of technological advancement increases, researchers have increasingly become the initiators of improved solutions to old problems (improved benefit / cost ratio for the end-user, improved safety or greater practicality in application, storage, and distribution). Irrespective of the origin, the development of the full product should be a collaborative exercise, steered towards an "end point" (a viable marketable product) by the three specialist teams.

The responsibility for the first decision point (D.P.1) lies with the marketing team. This is to assess whether the potential offered by the research results will provide a justifiable return on investment. This is directly related to the size and makeup of the overall market, and the expected ability of the resultant product to displace or compliment products already available.

In the case of the three research results under review, the decision to enter the original scientific research phase pre-empted studies on the size and nature of the heartwater control commercial market in the Southern African region. Later project-funded economic studies by B. Minjauw, etal. and Mukhebi, et al. in nine countries of the SADC region, estimated the HW impact costs avoided by vaccination. Although not truly marketing studies, they provide useful initial data for commercial market evaluations. (See paragraph A2 for a brief summary of these studies.)

D1b. Research - Proving the Concept

Typically, within the framework of a private sector company, the decision to take the initial concept to the commercial research phase, places the research team in the foreground. Their responsibility is to establish whether or not the concept is well founded and will in fact result in a product that will provide the market with the solution it requires. Research at this point is generally concentrated in two areas:

- The establishment of the methodology for the commercial production of the product according to Good Manufacturing Practices (GMP) standards. This includes the development of quality assurance standards or benchmarks. This level of standardization is critical at this point in order for the product to be able to be meaningfully moved to the "Development" stage and ultimately be registered.
- The setting up of both laboratory and field trial studies to establish the product's effectiveness in meeting the standards set out in the initial objectives. These include among others:
  - efficacy in target animals
  - the effect of factors such as breed, species, age, location (strain) on efficacy
  - the level of cross protection
  - the duration of effect or control

Only at a point where these attributes of the potential product have been established, and exhibit the characteristics which meet the markets requirements, can the decision as to whether or not to proceed to the Product Development phase be taken with any degree of confidence – Decision point 2. Based on the review of the status of the three research results in Part B of this report, all currently fall within this research phase, and require the completion of a number of planned studies. The research results are positioned at different points along the research continuum and fall short of this second decision point.

(Refer to Figure D2.). This fact, while not necessarily inhibiting an expression of interest in any of the discoveries from a commercial company, nevertheless is likely to decrease the chances of receiving a full commitment either in capital or other resources to form some type of partnership.

D1c. Product Development - Preparation for Registration

It is the responsibility of the Product Development Team to prepare the potential product for registration. This requires close collaboration with the research team, the production / manufacturing team and the marketing team. In broad terms this process has three main objectives (See Annex B.)

- To establish and document those properties of the product, which relate to or have the potential to impact on the safety of the target animal, consumers, people applying or handling the product and the environment. These include:
  - Pharmacology and toxicology studies, including acute, subacute and chronic toxicity tests
  - Residue data (where applicable)
  - Efficacy data
- To document in detail each step of the manufacturing process. These include among others detail of the:
  - Manufacturing Facility
  - Standard operating procedures
  - Validation data on instruments, analytical procedures, and production processes
  - Production and purification procedures
  - Manufacture of finished formulated product
  - In process assays performed on bulk active material, and formulated product
  - Stability data studies on both bulk active material and formulated product.
- To draw up the final product label, detailing valid claims, recommendations for use, administration / application, withdrawal periods, shelf life and storage conditions etc.

The stringency with which registration requirements are applied varies considerably with in the southern African region. However in order to gain entry into the South African, Zimbabwean and Botswanan markets, registration documentation comparable to that of an international (EU or US) standard is required. It is only on successful completion of the development / registration phase that a product is then taken to the final phase in the sequence – "Preparation for Market Launch" (Decision point 3)

As indicated in paragraph D1b. none of the three research results has yet progressed to the "Development" phase. As a result, a commercial company committing to any of the potential products at this point will consider not only the cost and resources required to complete this phase, but also the risk that results of the development studies may preclude it from final registration. Therefore, any formal commitment of capital and resources from a commercial company, is likely only to be given on the basis that the University of Florida's research results prove conclusively that the potential commercial product has the promise to displace, or at least compliment existing HW control products, to the level where the return on investment is secured.

D1d. Preparation for Product Launch

At the point at which it has been established that the conditions for registration by regulatory authorities have been met, the pathway to the commercialization of a product enters the final phase prior to launch (Figure D1). The key objective here is to establish a "position" for the product in each of the targeted market segments. Referring to the three

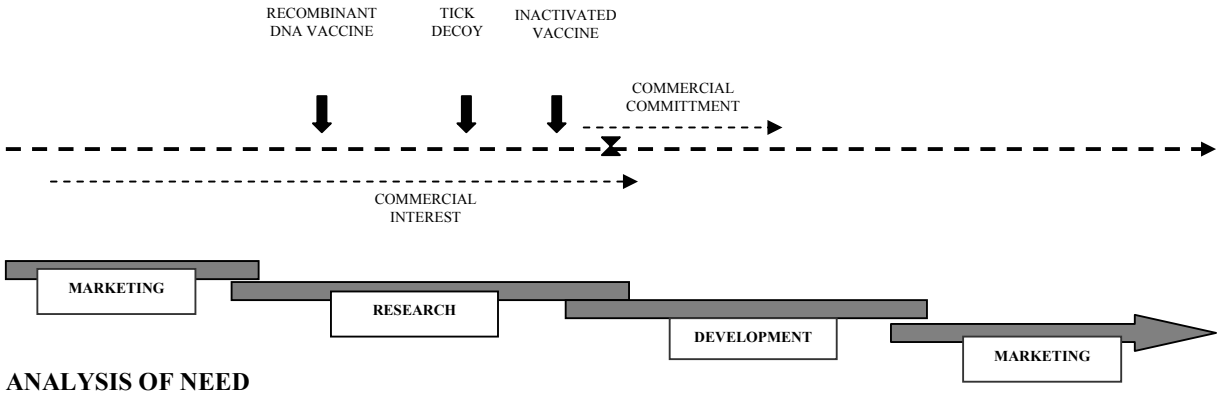
research results under review, this process cannot be carried out with any degree of accuracy. Any market positioning is therefore speculative and based on assumed results in both the current research and any subsequent development findings.

D2. Current Status and Speculative Market Positioning of the Products

Based on the review of the current status of the research on each of the potential product results (Refer to Part B), the objective here is to compare the results with the model set out in paragraph D1 above. Using this benchmark the three potential products have been overlaid on the product evolution continuum, in order to establish their position relative to the minimum point of interest and commitment from a commercial company. (Refer to Figure D2.) Additionally, based on the research results achieved so far, varying levels of unqualified interest or initial explorations into all three have been expressed by a number of commercial companies. In preparation for open discussions designed at facilitating the transfer of the discoveries to the commercial sector, this section also lists the remaining areas of scientific research required to enable the commercial companies to make an "informed commitment".

Finally this part examines the speculative "positioning" of the individual scientific results in the control of heartwater in southern Africa. (This is based on the assumption that the potential products achieve a marketable status.)

Figure D2. Schematic Representation of the Positioning of Results Relative to the Point of Commercial Commitment.



ANALYSIS OF NEED

- Identify problem
- Determine size of market
- Formulate concept

PROOF OF THEORY/CONCEPT

- Production methodology
- Efficacy in target animal
- Cross protection
- Duration of immunity

PREPARATION FOR REGISTRATION

- Production procedures [GMP]
- Patent registrations
- Stability data
- Pharmacology
- Toxicology data
- Residue data
- Establish claims and label

PREPARATION FOR LAUNCH

- Commercial literature
- Packaging
- Interest groups meetings
- Set up of distribution pathway
- Launch

D2a. Inactivated Heart Water Vaccine

Research carried out to date by the University of Florida team has already displayed a level of efficacy within target animals (60 – 70%) which has resulted in an interest being expressed by a number of commercial companies, including Intervet (Netherlands), American Home Products (USA) and Merial (France). However, as indicated in paragraph B1a, key elements of research, required to fully characterize the vaccine, remain to be completed. These include:

- Testing of the five additional adjuvants, for their effect on efficacy
- The determination of the optimal age for vaccination
- Trials to establish the duration of immunity

The results of these tests together with the existing findings could provide commercial companies with sufficient information on which to make an informed decision on whether or not to commit to developing and marketing the inactivated vaccine.

In the event that the inactivated HW vaccine is taken to the point of commercialization, it is likely to be positioned as a product for use in parallel with strategic acaricide tick control measures, promoting the development of endemic stability of the disease within heartwater areas. Additionally, vaccination of unexposed animals in non-heartwater areas prior to their movement into infected areas would decrease the mortality and production losses previously associated with these moves. The availability of a suitable vaccine would almost certainly replace the risky and impractical "infection and treatment" method currently employed in the areas of South Africa most severely challenged by HW. (See paragraph A3.)

While being targeted at both the commercial and small holder sectors, the requirement for the transport and storage of the vaccine under refrigerated conditions may impose some limit on the extent to which it can be used by small holders in more remote areas. Secondly, the existence of a higher degree of endemic stability of the disease in small holder areas may ultimately contribute to the relatively lower demand for the vaccine in his sector, except probably for young animals.

D2b. Recombinant DNA Heartwater Vaccine

Paragraph B2. of this report makes it clear that considerable advances in the development of a recombinant DNA vaccine and associated technologies have been made. However, unlike the inactivated heartwater vaccine, efficacy trial work has yet to show significant protection against heartwater in target animals. As a result, interest in the product from the commercial sector is less robust. To date Bioclones (Pty) Ltd. in South Africa appear to be the only company to have indicated an interest in evaluating the research data. While stating that the proof of efficacy in target animals is the point at which more conclusive discussions on this interest could be initiated, Bioclones further provides a list of additional data required on which to base a decision on commercial commitment for licensing agreements. (See Annex B).

If efficacy is shown for the recombinant DNA vaccines, conceptually two possible scenarios would ensue. First, as a stand-alone HW vaccine the recombinant DNA product would likely displace any inactivated HW vaccine with comparable efficacy, existing at that time. This displacement of the inactivated product is based on the assumption that the recombinant product would have greater cross protection properties and would be able to be transported and stored at ambient temperatures. In this case the positioning in the market would be as described for the inactivated product in paragraph D2a. Secondly the potential exists for a "cocktail" recombinant DNA vaccine that not only carries a wider spectrum of *C. ruminantium* strains, but also provides protection

against the most economically important tick borne diseases in southern Africa. A vaccine offering this scope of protection would have a profound beneficial effect on future strategies employed in the control of the major tick borne diseases including heartwater. Most notable of these would be a significantly reduced reliance on acaricides, with their associated problems as discussed in Part A.

D2c. Tick Decoy

As described in paragraph B3, research carried out on the tick decoy included efficacy trials that have shown a high degree of control of the Amblyoma tick species (>95%). Control of other economically important tick species has not been studied yet. Based on the results against Amblyomma ticks an interest in the product has been expressed by two commercial companies, these being Compressed Air Engineering in Zimbabwe and IPM Technologies in the USA.

In order to enable a commercial company to commit to this innovation, the most immediate task of project research is to prove that it (tail tag and collar), effectively controls all important tick species under high challenge conditions. (Ticks should be present at all predilection sites on the control animals, including the head, body and tail areas). This would require that the field trials, be conducted between the months of October and April in both Zimbabwe and South Africa. These trials would also need to establish the duration of efficacy.

It must be noted that in order to be accepted by registration authorities, the trials will ultimately have to be repeated by a commercial company, using the decoys manufactured under GMP for commercial use. As with the two heartwater vaccines, commitment from any commercial company to develop and market the tick decoy would require substantial capital resources. At the outset, prior to undertaking any registration field trial work, the commercial manufacturing plant would need to be established and financed. Additionally the commercial products from this plant would then need to be analyzed and documentation prepared for registration. It is therefore unlikely that a commercial company would receive a return on their investment with in the first 18 – 24 months. These factors would be relevant in negotiations with potential commercial partners.

Conceptually, if the tick decoy is developed and marketed, while being targeted at both the small holder and commercial farm sectors, it is likely to have greatest impact on the small holder sector. It would be positioned as an alternative to existing and frequently, financially unsustainable tick control methods (dip tank, spray, and pour-on). As well as environmental benefits, potentially there are many practical benefits to this method of tick control, including:

- Significant reduction in tick control costs
- Significant reductions in facility maintenance
- No reliance on large quantities of water in diptank application
- The need to have consistently accurate concentrations of the acaricide would not be a problem
- Distribution and storage of the decoy would be both simpler and safer than for conventional acaricides

D3. Concluding Comments on Commercial Potential

It is essential to recognize that the assessments and observations expressed in this section have been carried out against the backdrop of a model for the commercialization of products. The use of a model, while providing a very useful platform for evaluation and comparison, also implies a certain degree of rigidity, and tends to discount some of the uniqueness of an individual company's perspective on the value of the research to them.

The obvious need for improved methods of heartwater control has stimulated expressions of interest in the three research results from a number of companies. The intensity of this interest is a function of the degree to which the research has progressed along the model continuum. Actual interest or commitment would most accurately be determined through direct meetings with individual companies that have expressed initial interest. The aim of these meetings would be to assess the degree of interest and establish arrangements to effect the transition of the scientific research to the private sector under agreed upon terms for licensing or partnership.

SECTION III – CONCLUSIONS

1. Project Accomplishments Impressive

In the process of their research, project scientist have made major advances in understanding the molecular biology of the complex HW organism, trained several post graduates and published 169 manuscripts which greatly contribute to the body of scientific knowledge.

2. Significance of Research Results

Upon successful commercialization, the results of the HW research project would be of great value to both commercial and smallholder livestock production in SADC region through preventing HW production losses and reducing acaricide costs. Also this reduction in the present levels of acaricide use would be very environmentally beneficial.

3. Diagnostic Technology – Importance

Accomplishments in diagnostic test method technology provided invaluable tools useful within the region and elsewhere, including the U.S

4. Harare Lab Offers Important Capability

The HW research facilities at Harare are comprised of a wide range of valuable skills and resources. They are an exceptionally valuable asset for Zimbabwe and the region.

5. Lack of Focus on Commercialization

The University of Florida HW research, while very impressive, has not been sufficiently focused on commercialization of its research results. Little specific expertise or emphasis in product registration was available to the project, at least until quite recently.

6. Studies Needed to Make Results Attractive to Private Sector

The HW project results have not yet reached the point of being attractive for takeover by the private sector:

a. The inactivated vaccine is marginally efficacious and several critical studies remain to be completed including new adjuvants, cross protection and potency assay methods.
b. The DNA vaccine is at an earlier stage of research and trials on the efficacy in livestock are presently underway.
c. The tick decoy will be of low commercial interest until the planned field trial shows effectiveness against all important tick vectors, and possibly the tsetse fly.

7. Options for Future USAID Support

USAID is facing several options for decisions on future funding of the HW research project.

a. Terminate all ongoing project research as of April 30, 2001.
b. Support continuation of adjuvant trials and cross protection studies on the inactivated vaccine; continuation of efficacy trials in cattle and sheep on the DNA vaccine; and a small preliminary study of the tick decoy using currently available acaricides. Extend funding through September 2001 to accomplish this work.
c. Support a large-scale field trial of the tick decoy in South Africa and Zimbabwe during the 2001-2002 tick season. This trail is considered essential to eventual commercialization of the product. The additional cost of the trial would be quite low, but some level of activity at UF and at the laboratory in Harare would need to be maintained to support the trial. Funding would need to be continued through the fiscal year ending September 2002.
d. In addition to Option c, funding of duration of vaccine immunity trials during the same time period is an option. These trials are scheduled to start in June-July 2001 using the optimal adjuvant(s) in Zimbabwe and would have to continue through May-June 2002 to be completed.

SECTION IV – RECOMMENDATIONS

1. USAID should initiate efforts to seek SADC governments' and other agencies' financial support to maintain the operation of Veterinary Research Laboratory in Harare to serve animal agriculture in the SADC region. This asset would be valuable in future animal disease research and as a reference laboratory for various animal health activities such as those required to resolve sanitary-phytosanitary (SPS) issues related to agricultural trade among southern African countries.

2. Grant extension of the project to 30 September 2001 to allow completion of studies which will enhance attractiveness of project discoveries to private sector.[1]

3. Because of the potentially significant benefits the tick decoy technology holds for all of Africa, the trials planned for this next tick season should be given as much support as possible, unless negotiations with private sector lead to takeover of this research.

4. In order to assess and explore the attractiveness of the inactivated vaccine to commercial firms, Task Order 4.2 should initiate and fund negotiation meetings in the near future between UF staff and high-level officials of veterinary biologic companies such as: Intervet, American Home Products and Merial. USAID should play a facilitator role in these arrangements.

5. Depending on the results of meetings with the private sector regarding the need for scientific research to document the duration of immunity of the inactivated vaccine in order to make it attractive for commercialization, support of this research may deserve serious consideration by USAID for the 2001-2002 period.

1 This extension to September 2001 was granted by USAID to UF on April 19, 2001.

ANNEX A – HEARTWATER DISEASE[1]

Heartwater (HW) is an acute noncontagious infectious disease of ruminants affecting cattle, sheep, goats and antelope and is caused by the rickettsial organism *Cowdria ruminantium,* which is transmitted by ticks of the genus *Amblyomma*. The disease is characterized by fever, nervous signs, hydropericardium, hydrothorax, ascites, edema of the lungs and high mortality. In some wild ruminants the agent causes subclinical infection. The name "heartwater" is derived from the hydropericardium, which is commonly seen with this disease.

The disease is caused by *Cowdria ruminantium,* a rickettsial agent. It is the only species of the genus *Cowdria*, in the tribe Ehrlichia, family Rickettsiaceae, order Rickettsiales. The organism multiplies in vascular endothelial cells throughout the body and in the reticulum cells of the lymph nodes. The HW organism is extremely fragile and cannot persist outside of a host for more than a few hours. Because of its fragility, the organism must be stored in dry ice or liquid nitrogen to preserve its infectivity.

Hosts

Heartwater causes severe disease in cattle, sheep, goats, and water buffalo; mild disease in some indigenous African breeds of cattle, sheep and goats; and inapparent disease in several species of antelope indigenous to Africa. The blesbok (*Damaliscus albifrons*), the black wildebeest (*Connochaetes gnu*), the eland (*Taurotragus oryx oryx*), and the springbok (*Antidorcas marsupialis*) have experimentally been shown to be susceptible to HW, and although the natural disease in these animals is usually mild, deaths in springbok have been attributed to HW. The blesbok and wildebeest are known carriers of *C. ruminantium* and are believed to play a role in the maintenance of the disease in nature. Nonruminant hosts of *C. ruminantium,* such as guinea-fowl, leopard tortoises, and scrub hare, may also be important in the maintenance of the organism in nature because they are all known carriers of the agent.

In the United States the most common deer species, *Odocoileus virginianus* (the white-tailed deer), has been shown by experimental inoculation to be susceptible to *C. ruminantium*. Severe clinical signs were noted along with typical postmortem lesions. The mortality rate was high. *Amblyomma maculatum*, an experimentally proven vector of HW, is a common parasite of white-tailed deer in the southern United States.

Geographic distribution

Heartwater occurs only where vector ticks of the genus *Amblyomma* are active. For decades, the disease has been known to occur in most countries of Africa south of the Sahara Desert and on the island of Madagascar. For the last half century or more, the disease has been considered one of the most important livestock diseases in Africa and has been surpassed in importance only by trypanosomiasis and East Coast fever in some areas.

Heartwater is now known to occur in the Caribbean, where the vector tick *A. variegatum* (tropical bont tick) has been recognized for many years. This tick, now known to occur on numerous Caribbean islands (e.g. Puerto Rico, Antigua, Guadeloupe, Martinique, St. Lucia, Nevis, St. Kitts) was probably introduced to the French Antilles with a shipment of cattle from Senengal in the 1830's. A fatal disease of cattle with neurologic and hemorrhagic signs, which in retrospect could have been HW, was described from Guadeloupe in 1932. Rapid spread of the tropical bont tick in the West Indies is believed to have occurred only after the introduction of the

1 This annex is excerpted and revised from the writings of Dr. John Mare′ in the USAHA Foreign Animal Diseases Manual, 1998.

cattle egret from Africa in the early 1960's. Egrets are now known to be efficient porters of the tick. Heartwater has been diagnosed on the islands of Antigua, Guadeloupe, and Marie Galante. *Cowdria ruminantium* is far more widespread in the Caribbean than was formerly believed. Recent serological surveys have demonstrated HW antibodies in cattle from 10 Caribbean islands (Antigua, Dominica, Granada, Guadeloupe, Martinique, Montserrat, St. Kitts, St. Lucia, St. Martin, and St. Vincent).

Transmission

Heartwater is transmitted only by ticks of the genus *Amblyomma*. Of the 12 species known to transmit the disease, *A. variegatum* (tropical bont tick) is by far the most important, for it is widely distributed in Africa and has extended its range to include Yemen, Reunion, the Cape Verde islands, and several islands of the West Indies (26). Other major vector species are the bont tick *A. hebraeum* (in southern Africa), and *A. lepidum* (in East Africa and the Sudan). *Amblyomma astrion* (mainly on buffalo) and *A. pomposum* are also natural vectors of the disease, and five other African ticks – *A. sparsum, (*the large reptilian tick), *A. gemma, A. cohaerans, A. marmoreum, (*the tortoise tick), *and A. tholloni* (the elephant tick)- have experimentally been shown to be capable of transmitting HW. Scientists at the University of Florida have recently found the *A. marmoreum* and the *A. sparsum* ticks on the tortoises imported to Florida from Africa. They found that a batch of *A. sparsum* tested positive for having been exposed to the HW organism. Both of these ticks will feed on cattle, sheep and goats in their larvae and nymph stages but not commonly. Adults prefer tortoises.

Two North American species of *Amblyomma* ticks have been shown to be capable of transmitting the disease. They are *A. maculatum* (the Gulf Coast tick) and *A. cajennense*, but neither of these ticks has been incriminated as natural vectors of HW. The former tick is widely distributed in the eastern, southern and western United States, and was shown to be as good a vector as one of the principal African vectors, *A. hebraeum*.

Amblyomma ticks are three-host ticks whose life cycles may take from 5 months to 4 years to complete. Because the ticks may pick up the infection as larvae or nymphs and transmit it as nymphs or as adults, the infection can persist in the tick for a very long time. The infection does not pass transovarially. *Amblyomma* ticks are multihost and will feed on a wide variety of livestock, wild ungulates, ground birds, small mammals, reptiles, and amphibians.

Signs

The incubation period is generally shorter in sheep and goats than cattle. Experimental intravenous inoculation usually results in a febrile response between the 7^{th} and 10^{th} day after inoculation in sheep and goats, and between the 10^{th} and 16^{th} day after inoculation in cattle. Under field conditions, susceptible animals can be expected to show signs of the disease 14 to 28 days after introduction into an HW–infected area.

Heartwater occurs in different clinical forms determined by variation in susceptibility of the hosts and the virulence of various strains of the HW agent.

-- The peracute form of the disease is usually seen in Africa in nonnative breeds of cattle, sheep and goats introduced to an HW enzootic area. Heavily pregnant cows are especially prone to develop the peracute disease. Sudden death occurs, usually preceded only by a fever, severe respiratory distress, and terminal convulsions. Severe diarrhea may be seen in some breeds of cattle (e.g. Jersey, Guernsey).

-- The acute form of the disease, by far the most commonly observed syndrome, is seen in nonnative and indigenous domestic ruminants. A sudden fever of up to 107° F (42° C) is followed by inappetance, depression, listlessness, and rapid breathing. Nervous signs then develop, the most prominent being chewing movements, twitching of the eyelids, protrusion of the tongue and circling, often with highstepping gait. The animal may stand with its legs apart

and head lowered. The nervous signs increase in severity, and the animal goes down in convulsions. Galloping movements and opisthotonos are commonly seen before death. Hyperesthesia is often observed in the terminal stages of the disease, as is nystagmus and frothing at the mouth. Diarrhea is occasionally seen, especially in younger animals. The acute disease is usually fatal within a week of the onset of signs.

-- Rarely, the disease may run a subacute course characterized by prolonged fever, coughing (a result of lung edema), and mild incoordination; recovery or death occurs in 1 to 2 weeks. A mild or subclinical form of the disease, known as "heartwater fever", is seen in partially immune cattle or sheep, in calves less than 3 weeks old, in antelope, and in some indigenous breeds of sheep and cattle with high natural resistance to the disease. The only clinical sign in this form of the disease is a transient febrile response.

Once signs of the disease have developed, the prognosis is poor for nonnative sheep, goats, and cattle infected with the more virulent strains of the HW organism. The mortality rate in merino sheep may be 80 percent in contrast to 6 percent mortality observed in Persian or Afrikander sheep. Angora goats are extremely susceptible to HW. In susceptible cattle, mortality of about 60 percent is not uncommon. Apparently maternal antibody protection is not always effective against HW as many farmers report sickness and death in sheep and goats at 2-3 weeks age and in calves at 2-3 months age. This leads farmers to practice preventive measures on sheep and goats as young as a few days and on calves as young as three weeks.

Lesions

The gross lesions in cattle, sheep and goats are very similar. Heartwater derives its name from one of the prominent lesions observed in the disease, namely pronounced hydropericardium. The accumulation of straw-colored to reddish fluid in the pericardium is more consistently observed in sheep and goats than in cattle. Ascites, hydrothorax, mediastinal edema, and edema of the lungs, all resulting from increased vascular permeability with consequent transudation, are frequently encountered. Meningeal congestion and edema are often present. Brain congestion may occur, but brain lesions can be remarkably few when one considers the severity of the nervous signs observed in this disease.

Diagnosis

Field Diagnosis

The presence of *Amblyomma* ticks plus the rather characteristic signs and lesions of HW allows tentative field diagnosis of the disease, which must then be confirmed by demonstration of the causative organism, its antigens, or its DNA.

Laboratory Diagnosis

1. Demonstration of the Organism: The HW organism stains purplish-blue with Giemsa stain and can be seen by microscopic examination of brain smears prepared as follows: A small piece of cerebrum, cerebellum, hippocampus, or other well-vascularized portion of the brain is macerated between two microscope slides. The resultant pulp is then drawn across a slide with varying pressure, which results in "ridges and valleys" on the slide. The slide is then air-dried, fixed with methanol, and stained with Giemsa. Under low magnification, the capillaries will be found extending from the "thick" areas of the slide. Examination of the capillary endothelial cells under oil immersion will reveal the blue to reddish-purple clumps of organisms. The HW organisms can also be observed in smears prepared from the intima of large blood vessels or in stained sections of kidney glomeruli and lymph nodes.

Although microscopic examination of Giemsa-stained brain smears is still widely employed in HW diagnosis, newer and more sensitive techniques such as the se of DNA probes have been applied to detect *Cowdria* nucleic acids in tissues of infected livestock and ticks.

These newer techniques should supplant the older methods of diagnosis as facilities and equipment become more available in HW-endemic areas.

2. Antibody Detection: The indirect fluorescent antibody (IFA) test has extensively been used for HW antibody detection, and the newer competitive enzyme-linked immunosorbent assay (CELISA) promises to be a useful addition to the meager array of tests available for the detection of HW antibodies. The cross-reactions described with several *Ehrlichia* spp. can now be eliminated with the use of more specific antigens and monoclonal antibodies.

Differential Diagnosis

The peracute form of HW can be confused with anthrax. The acute nervous form of HW can be confused with rabies, tetanus, chlamydiosis, bacterial meningitis or encephalitis, cerebral trypanosomiasis, piroplasmosis or theileriosis, and various intoxications such as with strychnine, lead, organophosphates, or chlorinated hydrocarbons. Heavy helminth infestations may produce accumulations of fluid similar to those seen in HW. Arsenical poisoning may resemble the enteric form of the disease, and certain poisonous plants may produce signs and lesions similar to those seen in HW.

Treatment

Tetracycline antibiotics (especially oxytetracycline) are very effective in the treatment of HW, especially when animals are treated early in the course of the disease. Tetracycline antibiotics administered to young animals and naïve animals being moved into "HW areas" before signs appear will suppress the disease entirely, but will allow immunity to develop.

Immunity

Animal recovering from the natural disease or from artificial exposure to the organism are solidly immune for a variable period ranging from 6 months to 18 months. Animals exposed to reinfection by tick challenge during this period of resistance will have their immunity reinforced and will remain immune as long as they are periodically reinfected. There is now conclusive evidence that immunity to HW is T-cell mediated and that circulating antibodies play a minor role in immunity.

Control

Tick Control

The HW organism is extremely fragile and cannot persist outside of a host for more than a few hours. The principal mode of bringing the disease into an area is thus through introduction of infected ticks or carrier animals. It is not known for how long wild or domestic ruminants can be a source of infection for ticks in nature. It has been shown that experimentally infected sheep, cattle, and African buffalo can be a source of infection for nymphs of the bont tick (*A. hebraeum)* for 223, 246, and 161 days, respectively. After molting to adults, the ticks transmit the disease to susceptible sheep. This prolonged carrier state needs to be considered when animals are moved from HW-enzootic to HW-free areas. It is also not known for how long a tick can remain a carrier of the organism. Careful dipping and hand-dressing with acaricide preparations followed by inspection to ensure the absence of ticks is recommended for animals in transit to HW free areas.

Vector control measures aimed at eradication of *Amblyomma* ticks by dipping of cattle have failed principally because the vector is a multihost tick with a high rate of reproduction. The development of acaricide resistance has further complicated attempts at tick control. In

enzootic areas, tick levels are now allowed to remain at levels high enough to permit reinfection of immune animals to booster the immunity.

Control by Treatment

Cattle, sheep, and goats moving into an HW-enzootic area can be protected from HW by prophylactic treatment with tetracycline (short or long-acting) either by feeding or by inoculation soon after they arrive to the enzootic area and are challenged by amblyomma ticks. However, they should be kept under surveillance and individually treated if clinical signs are seen.

Artificial Immunization Control

Calves and lambs are resistant to *C. ruminantium* in the first 1-4 weeks of life. This resistance seems to be true age resistance and has successfully been used in the immunization of cattle and sheep. Calves of less than 4 weeks of age, and lambs in the first week of life can be immunized by intravenous inoculation of HW-infected blood. The infection that follows is usually mild, and upon recovery animals are immune to reinfection because immunity is continuously stimulated by natural exposure to the organism. Older animals or very valuable calves should be examined daily after immunization and should be treated with antibiotics as soon as the febrile response commences. A subcutaneous implant of doxycycline at the time of immunization will eliminate the labor-intensive tetracycline treatment method. The immunity will not be affected by the antibiotic treatment. Flock immunization of sheep and goats can be accomplished by inoculation followed by mass treatment at the end of the incubation period.

Immunologically different strains of the organism do exist, but present evidence indicates that there is considerable cross-protection between different strains, thus allowing successful immunization. However, there are some strains between which there is little cross-protection.

A strain of *C. ruminantium,* attenuated by serial passage in bovine umbilical endothelium cells has been shown to confer solid HW immunity to sheep and goats. This finding suggests that a live-attenuated vaccine to HW may soon be available, but because other strains of the organism have not become attenuated by cell-culture passage, the degree of cross-protection between strains still needs clarification.

ANNEX B - STANDARD BIOLOGIC PRODUCT RESEARCH AND DEVELOPMENT PROCEDURES[1]

1. Technical disclosure, under cover of a confidentiality agreement, of the structure of the DNA vaccine and description of the method of production, purification and yield of the vaccine, including sequence data.

2. Release specifications for the active ingredients and formulated product.

3. Manufacturing facility
   - SAP's , SOP's and validation data on instruments, analytical procedures and production process.
   - Description of the primary production and purification areas.
   - Bank of genetic materials and storage thereof.

4. Production and Purification Procedures
   - Equipment used in production
   - Chemicals used in production
   - Master batch records
   - Cleaning procedures
   - Energy consumption

5. Manufacture of Finished Formulated Product
   - Procedures and master batch record

6. In-process and Finished Product Testing
   - Assays performed in-process
   - Assays performed on bulk active
   - Assay performed on formulated product

7. Stability Data
   - Bulk active
   - Formulated product

8. Pharmacology and Toxicology
   - Acute, sub-acute and chronic toxicity studies
   - Data on the ability of the vaccine to produce antigenic expression in target host cells

9. Field Studies in at least one target animal showing efficacy and duration of immunity

10. A double blind placebo controlled study to indicate the percentage of animals showing immunity and its duration.

1 This outline was provided to the team by Dr. Cyril Donniger of Bioclones (PTY) Limited, South Africa. It refers to DNA vaccine primarily but in general applies to any biologic product. At some early point in the process, licensing agreements would undoubtedly be signed between the stakeholders in the development.